PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

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PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	21.12.99
Applicant's or agent's file reference FB/SD/B45110	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP98/06040	17/09/1998	26/09/1997

Applicant
SMITHKLINE BEECHAM BIOLOGICALS S.A. et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

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PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
FB/SD/B45110		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP98/06040	17/09/1998	26/09/1997
International Patent Classification (IPC) or national classification and IPC		
C12N15/49		
Applicant		
SMITHKLINE BEECHAM BIOLOGICALS S.A. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
17/04/1999	21.12.99
Name and mailing address of the international preliminary examining authority:	Authorized officer
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Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP98/06040

I. Basis of the r port

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-26	as originally filed			

Claims, No.:

1-31	as received on	08/12/1999	with letter of	06/12/1999

Drawings, sheets:

1-9	as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-31
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-24, 27-31
	No:	Claims	25, 26
Industrial applicability (IA)	Yes:	Claims	1-31
	No:	Claims	

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

1. Reasoned statement

1.1. The application describes fusion proteins consisting of a fragment of H. influenzae protein D and HIV Tat and/or HIV Nef proteins. The molecules produce immune responses in mice and inhibit T-cell activation in vitro.

1.2. Amended claims (Art. 34(2)(b) PCT)

The IPEA does not find a clear basis in the application documents as originally filed leading directly and unambiguously to the subject matter of claims 13 and 18. While there is an example specifying carboxymethylation of a Nef-Tat fusion protein, there seems to be no basis for the carboxymethylation to be applied to all the vaccines. Equally, there are references to oil in water emulsions on p. 8 and p. 23 of the application documents, but they are more specific in that they mention the presence of tocopherol and squalene. Strictly speaking, there is no basis for merely water in oil.

1.3. Novelty (Art. 33(2) PCT)

The new set of claims refers to vaccine compositions comprising HIV Tat and/or Nef fusion proteins (claims 1-19), Nef-Tat or Tat-Nef fusion proteins (claims 20-24), methods of preparing Nef or Tat in P. pastoris (claims 25-29), and a vaccine comprising recombinant Tat-containing protein (claims 30-31). All these objects have not been mentioned in the cited prior art and are therefore novel.

1.4. Inventive step (Art. 33(3) PCT)

Tat-Nef or Nef-Tat fusion proteins as well as the use of Nef or Tat proteins fused to additional proteins for vaccination are not mentioned in the prior art. Therefore, the subject matter of claims 1 to 24, and 27 to 31 cannot be derived from the cited prior art in an obvious way.

Claims 25 and 26 are considered to lack inventive step. HIV Nef and Tat proteins have been known and expressed (see the documents cited in the ISR). P. pastoris is a commonly used host for recombinant protein expression which presents an

obvious alternative to other frequently used hosts. Unless the applicant demonstrates unexpected properties of the expressed protein (when compared to the proteins of the prior art) or the non-obvious solution of problems encountered when expressing the protein in P. pastoris, claims 25 and 26 are considered to lack inventive step.

2. Certain observations

2.1. The terms "Tat" and "Nef" are apparently not used to designate the full-length proteins only (see claims 1 and 9 or 10). Thus, they are used in an arbitrarily defined way and render the scope of the claims unclear.

2.2. Several claims refer to "derivatives". This term is open to interpretation and as such renders the scope of the respective claims unclear.

2.3. The term "fusion partner" is only supported as far as protein-protein fusions are concerned.

CLAIMS

1. A vaccine composition which comprises a protein comprising

(a) an HIV Tat protein or derivative thereof linked to either (i) a fusion partner or (ii) an HIV Nef protein or derivative thereof; or

(b) an HIV Nef protein or derivative thereof linked to either (i) a fusion partner or (ii) an HIV Tat protein or derivative thereof; or

(c) an HIV Nef protein or derivative thereof linked to an HIV Tat protein or derivative thereof and a fusion partner,

in admixture with a pharmaceutically acceptable excipient.

2. A composition as claimed in claim 1 comprising a Tat-Nef fusion protein or derivative thereof.

3. A composition as claimed in claim 1 comprising a Nef-Tat fusion protein or derivative thereof.

4. A composition according to any one of claims 1 to 3 wherein the derivative of the Tat protein is a mutated Tat protein.

5. A composition according to any one of claims 1 to 4 wherein the derivative of the Nef protein is a mutated Nef protein.

6. A composition as claimed in any one of claims 1 - 5 wherein the fusion partner is a lipoprotein or derivative thereof.

7. A composition as claimed in claim 6 wherein the lipoprotein is Haemophilus Influenza B protein D or derivative thereof.

8. A composition as claimed in claim 7 wherein the fusion partner comprises between 100-130 amino acid from the N terminal of Haemophilus Influenza B protein D.

9. A composition as claimed in any one of Claims 1 to 8, wherein the Tat protein is the entire Tat protein.

10. A composition as claimed in any one of Claims 1 to 8, wherein the Nef protein is the entire Nef protein.

11. A composition as claimed in any one of Claims 1 to 10, wherein the Tat protein is fused to an HIV Nef protein and a fusion partner.

12. A composition as claimed in any one of claims 1 to 11, wherein the protein has a Histidine tail.

13. A composition as claimed in any one of claims 1 to 12 wherein the protein is carboxymethylated.

14. A composition as claimed in any one of claims 1 to 13, additionally comprising an adjuvant.

15. A composition as claimed in claim 14, wherein the adjuvant is a TH1 inducing adjuvant.

16. A composition as claimed in claim 14 or 15 which adjuvant comprises monophosphoryl lipid A or a derivative thereof such as 3 de-O-acylated monophosphoryl lipid A.

17. A composition as claimed in any one of claims 14 to 16 additionally comprising a saponin adjuvant.

18. A composition as claimed in any one of claims 14 to 17 which additionally comprises an oil in water emulsion.

19. A composition as claimed in any one of claims 1 to 18 further comprising HIV gp160 or its derivative gp120.

20. A protein comprising an HIV Tat protein or derivative thereof linked to an HIV Nef protein or derivative thereof in Nef-Tat or Tat-Nef orientation.

21. A nucleic acid encoding a protein of claim 20.

22. A host transformed with a nucleic acid of claim 21.

23. A host as claimed in claim 22 wherein the host is either *E.coli* or *Pichia pastoris*.

24. A method of producing a protein of claim 20, comprising providing a host as claimed in claim 22 or 23, expressing said protein and recovering the protein.

25. A method of preparing (i) an HIV Nef protein or derivative thereof or (ii) an HIV Tat protein or derivative thereof in *Pichia pastoris* which method comprises the steps of transforming *Pichia pastoris* with DNA encoding said HIV Nef protein or derivative thereof or HIV Tat protein or derivative thereof, expressing said protein and recovering the protein.

26. The method of claim 24 or claim 25 further comprising a carboxymethylation step performed on the expressed protein.

27. A method of producing a vaccine, comprising admixing the protein from any one of claims 24 to 26 with a pharmaceutically acceptable diluent.

28. The method of claim 27 further comprising the addition of HIV gp160 or its derivative gp120.

AMENDED SHEET

29. The method of claims 24 to 28 further comprising the addition of an adjuvant, particularly a TH1 inducing adjuvant.

30. A vaccine composition comprising a recombinant Tat-containing protein formulated with a mixture of 3D-MPL, QS21 and an oil in water emulsion

31. A composition as claimed in claim 30 wherein the oil in water emulsion comprises squalene, polyoxyethylene sorbitan monooleate and α-tocopherol.